referred to in the specification continued to be defective. Therefore, the Applicant's attorney, pursuant to the Office Communication, contacted the Examiner by phone on December 11, 2003 to discuss the specifications' deficiencies with respect to the embedded hyperlinks. A possible solution for removing the embedded hyperlinks was identified by the Examiner and subsequently communicated to the Applicant's attorney by voice mail, also on December 11, 2003. The Examiner has suggested that all of the hyperlinks referred to in earlier correspondence with the USPTO in this matter be further amended as indicated herein below. However, the Applicant is not entirely confident that the USPTO's recommended means for removing the embedded hyperlinks will result in all of the hyperlinks being removed, but in view of the Examiner's suggestion coupled with the amendments and remarks below, it is believed that this submission is fully compliant with and fully responsive to the Office Communication and to the earlier Non-Responsive Letter mailed May 30, 2003. Therefore, it is respectfully requested that the Examiner enter the amendments listed below and find that the specification and claims are now in condition for allowance. It is believed that this paper is timely filed within the granted non-fee one month from the date the Office Communication was mailed and that no fee is due with this response.

Amendments to the Specification

The Applicant requests that the previously requested amendments to the specification be disregarded in place of the Amendments to the Specification indicated herewith in the attached replacement paragraphs shown on the following several pages, which are marked up to show changes made relative to the specification as filed. Text or markings to be added are shown as underlined text or markings, and text or markings to be deleted are shown as text or markings having a line-through or strike-through. It is believed that no new matter is added to the specification with the amendment to the indicated replacement paragraphs.

Amendments to the Specification With Markings to Show Changes Made

*Please make the following changes as indicated in the paragraph bridging pages 2-3 in the specification as filed:

Bacillus thuringiensis is a spore-forming Gram-positive bacterium. During sporulation, *B. thuringiensis* produces proteinaceous inclusions which are composed of proteins known as insecticidal crystal proteins (ICPs), Cry proteins, or delta-endotoxins. These proteins are toxic to a variety of insect species including orders Lepidoptera, Coleoptera, Diptera, Hemoptera, Hymenoptera, Orthoptera, and Mallophaga and to nematodes, mites, and protozoa (Beegle and Yamamoto, *Can. Entomol. 124*:587-616; Feitelson, Advanced Engineered Pesticides (L. Kim, *ed.*), Marcel Dekker, Inc., New York (1993), pp. 63-71; Feitelson, *et al., Bio/Technology 10:*271-275; U.S. Patent No. 4,948,734 (1990)). Due to their high specificity for particular insect pests and their safety for man and the environment, ICPs have been used as biopesticides for the last three decades. Using molecular genetic techniques, numerous delta-endotoxin genes have been isolated and their DNA sequences determined. The cloning and sequencing of a number of δ-endotoxin genes from a variety of *B. thuringiensis* strains has been described and are summarized by Schnepf *et al.* (*Microbiol. Mol. Biol. Rev. 62*:775-806, *Bacillus thuringiensis* And Its Pesticidal Crystal Proteins, 1998). The nomenclature and appearance of newly identified genes is summarized and regularly updated by Crickmore, N., Zeigler, D.R., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J., Bravo, A. and Dean, D.H. through the "Bacillus thuringiensis toxin nomenclature" link at the University of Sussex Department of Biology web site with the extension biols.susx.ac.uk/home/neil_crickmore/bt/ ~~http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/~~. These genes have been used to develop certain genetically engineered *B. thuringiensis* products that are in commercial use. Recent developments have seen new δ-endotoxin delivery systems developed, including genetically engineered plants that contain and express δ-endotoxin genes. *Bacillus thuringiensis* is a key source of genes, which when modified can be used for transgenic expression to provide pest resistance in plants.

*Please make the following changes to the last full paragraph on page 11 in the specification as filed:

Open reading frames in genomic sequences can be screened for the presence of protein homologues utilizing a number of different search algorithms that have been developed, one example of which is the suite of programs referred to as BLAST programs. There are five implementations of BLAST, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology 12:*76-80 (1994); Birren *et al., Genome Analysis 1:*543-559 (1997)). Other examples of suitable programs that can be utilized are well known in the art. In addition, unidentified reading frames may be screened for by gene prediction software such as GenScan, which is located at the Stanford University web site with the extension gnomic.Stanford.edu/GENSCANW.html ~~http://gnomic.stanford.edu/GENSCANW.html~~. Novel genes, i.e., with no known homologs, can be predicted with the program GeneMark, which calculates the probability of a gene based on the presence of a gene-like 'grammar' in the DNA sequence (i.e., start and stop signals, and a significant open reading frame) and statistical analyses of protein-coding potential through biases in putative codon usage (see the Georgia Tech University web site with the extension genemark.biology.gatech.edu/GeneMark ~~http://genemark.biology.gatech.edu/GeneMark~~ for details).

*Please make the following changes to the paragraph bridging pages 13-14 in the specification as filed:

Another example of algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul et al, J. Mol. Biol. 215: 403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI) web site with the extension ncbi.nlm.nih.gov~~,~~ ~~http://www.ncbi.nlm.nih.gov/~~; see also Zhang, Genome Res. 7:649-656 (1997) for the "PowerBLAST" variation. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, J. Mol. Biol. 215: 403-410 (1990)). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions

along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-10919(1992)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The term BLAST refers to the BLAST algorithm which performs a statistical analysis of the similarity between two sequences; see, e.g., Karlin, Proc. Natl. Acad. Sci. USA 90:5873-5787 (1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

*Please make the following changes to the paragraph bridging pages 35 and 36 in the specification as filed:

Exogenous genetic material may be transferred into a plant cell by the use of a DNA vector or construct designed for such a purpose. Vectors have been engineered for transformation of large DNA inserts into plant genomes. Binary bacterial artificial chromosomes have been designed to replicate in both *E. coli* and *A. tumefaciens* and have all of the features required for transferring large inserts of DNA into plant chromosomes <u>as set forth in the protocol by</u> Choi and Wing, <u>available at the Clemson University web site with the extension genome.Clemson.edu/protocols2-nj.html</u> ~~**http://genome.clemson.edu/protocols2-nj.html**~~, July, 1998. ApBACwich system has been developed to achieve site-directed integration of DNA into the genome. A 150 kb cotton BAC DNA is reported to have been transferred into a specific *lox* site in tobacco by biolistic bombardment and *Cre-lox* site specific recombination.

*Please make the following changes in the second full paragraph on page 55 in the specification as filed:

It is well known to a person skilled in the art that the sequence data from a large scale shotgun sequencing project can be processed and assembled into contigs, which represent a reconstruction of the original chromosomal genome sequence from the cloned fragments. Programs are available in the public domain that can analyze the sequence output and assemble the sequences into larger sequence regions representing contiguous sequences of the target genome. Examples of such programs can be found at, for example, the Washington University website with the extension genome.wustl.edu/gsc, the Sanger University website with the extension sanger.ac.uk, and the University of Washington website with the extension mbt.washington.edu ~~http://genome.wustl.edu/gsc~~, ~~http://www.sanger.ac.uk~~, and ~~http://www.mbt.washington.edu~~. An example of a sequence reading program is Phred (which can be obtained at the University of Washington website with the extension mbt.washington.edu ~~http://www.mbt.washington.edu~~). Phred reads DNA sequencer trace data, calls bases, assigns quality values to the bases, and writes the base calls and quality values to output files.

*Please make the following changes to the third full paragraph on page 55 in the specification as filed:

The process of assembling DNA sequence fragments generally involves three phases; the overlap phase, the layout phase and the multi-alignment, or consensus, phase. In the overlap phase, each fragment is compared against every other fragment to determine if they share a common subsequence, an indication that they were potentially sampled from overlapping stretches of the original DNA strand. Pairs of fragments are compared in two ways; 1) with both fragments in the same relative orientation, and 2) with one of the fragments having been reverse complemented. In the layout phase, a series of alternate assemblies or layouts of the fragments based on the pairwise overlaps is generated. A layout specifies the relative locations and orientations of the fragments with respect to each other and is typically visualized as an arrangement of overlapping directed lines, one for each fragment. The general criterion for the layout phase is to produce plausible assemblies of maximum likelihood. In this manner, it can be determined if there is more than one way to put the pieces together and if different solutions appear equally plausible. In such a case, one would return to the lab and obtain additional information to resolve the ambiguity. The multi-alignment, or consensus, phase uses more information than just the

pairwise alignments in the layout. The sequences of all the fragments in a layout are simultaneously aligned, giving a final set of contigs representing regions of the target genome. An example of an assembly program is PHRAP, which can be found at the University of Washington web site with the extension chimera.biotech.washington.edu/UWGCG/tools/phrap.htm ~~http://chimera.biotech.washington.edu/UWGC/tools/phrap.htm~~.

*Please make the following changes to the second full paragraph on page 56 of the specification as filed:

Similarity analysis includes database search and alignment. Examples of public databases include the DNA Database of Japan (DDBJ) web site with the extension ddbj.mig.ac.jp ~~(http://www.ddbj.nig.ac.jp/)~~; the Genebank web site with the extension ncbi.nlm.nih.gov/web/Genbank/Index.html ~~(http://www.ncbi.nlm.nih.gov/web/Genbank/Index.htlm)~~; and the European Molecular Biology Laboratory Nucleic Acid Sequence Database (EMBL) web site with the extension ebi.ac.uk/ebi_docs/embl_bl.html ~~(http://www.ebi.ac.uk/ebi_docs/embl_db.html)~~. A number of different search algorithms have been developed, one example of which are the suite of programs referred to as BLAST programs. There are five implementations of BLAST, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology 12:*76-80 (1994); Birren *et al., Genome Analysis 1:*543-559 (1997)).

*Please make the following changes to the first full paragraph on page 63 of the specification as filed:

After the base calling is completed, sequence preprocessing is performed. Quality assessment and trimming is performed by determining the maximum scoring segment of PHRED quality score > 10. Cloning sequences are removed by utilizing cross_match, available at the University of Washington website with the extension mbt.washington.edu ~~(http://www.mbt.washington.edu)~~ and searching a database of relevant cloning sequences. Contaminating sequences (E. coli, yeast, vector, linker) are then removed from the dataset by utilizing cross_match to search a database of contamination sequences.

*Please make the following changes to the second full paragraph on page 63 of the specification as filed:

The preprocessed sequences are then assembled into contigs, or groups of overlapping sequences. Contigs are assembled using PHRAP (phragment assembly program), also developed by Green at the University of Washington <u>website with the extension mbt.washington.edu</u> ~~(http://www.mbt.washington.edu)~~ using default assembly parameters. This program takes a file of shotgun sequences and compiles consensus contig sequences. Alignments are influenced by quality scores, based on Green's algorithm. Singletons are the remaining sequences without sufficient overlaps with others after the assembly.